Fig. 3. SDS-PAGE of ammonium sulfate precipitate (AS), and active fractions from C3 and sulfopropyl (S1 and S2) HPLC separations of cucumber proteins.

Protein samples were concentrated, desalted, and run on SDS-polyacrylamide gels according to the method of Laemmli. Gels were stained with Coomassie Brilliant Blue R 250. Arrows indicate the major protein bands at 29 and 30 kD in the S1 and S2 fractions which appear to possess the extension-inducing activity.

Fig. 4. The effects of DTT, metal ions, methanol and water boiling, and protease treatments on reconstituted extension.

(A) The effect of DTT on reconstituted extension. Growing wall specimens of cucumber hypocotyl were inactivated by heat and then reconstituted by shaking in a solution of active C3 proteins (estimated concentration of 50 micrograms per mL). Walls were then clamped under constant load (as described in figure 1) firstly in a bathing solution of 50 mM Hepes, pH 6.8. After 20 min. the solution was changed for 50 mM sodium acetate, pH 4.5 (first arrow). After a further 40 min. DTT (from a 100 mM stock solution) was added to give a final concentration of 10 mM. The first two lines represent typical traces from 4 experiments with and without DTT addition.

For the effects of metal ions, growing wall specimens from cucumber hypocotyl were inactivated by heat and then clamped under constant load (as described in figure 1) in a bathing solution of 50 mM sodium acetate, pH 4.5. After 20 min. the bathing solution was exchanged for a fresh one containing 50 micrograms per mL of active C3

proteins (first arrow). After a further 40 min. $AlCl_3.6H_2O$ or $CuCl_2.2H_2O$ (from 100 mM stock solutions) was added to bring the bathing solution to a final concentration of 1 mM (second arrow). All experiments were repeated 4 times. Line 3 shows extension without the addition of metal ions, the next two lines show typical data obtained with the addition of $AlCl_3$ and $CuCl_2$ respectively.

For the effects of boiling cell walls in methanol or boiling in water on the recovery of extension inducing activity, growing cucumber hypocotyl tissue was first boiled for 3 min. in methanol or for 30 sec in distilled water, wall fragments were recovered, cleaned and extracted (as described in figure 1). Proteins were precipitated with $(NH_4)_2SO_4$) and resuspended in 50 mM sodium acetate, pH 4.5 before being tested in the reconstitution assay described in figure 1. Lines 6 and 7 are representative data from 4 experiments.

(B) Growing wall specimens were inactivated by heat and reconstituted with C3 proteins. Reconstituted walls were incubated with 1000 units of trypsin or chymotrypsin for 4 hr at 30°C, in 50 mM Hepes, pH 7.3, or with 2 milligrams per mL of pronase or papain for 4 hr at 30°C, in 50 mM sodium acetate, pH 5.0. Controls were reconstituted and incubated in the same manner without the addition of proteases. At the end of the incubations tissues were clamped under constant load (as described in figure 1), first in 50 mM Hepes, pH 6.8. After 30 min. the bathing solution was replaced by 50 mM sodium acetate, pH 4.5. The difference in the two rates of extension was calculated. Data presented are the means of four experiments in each case.